REMARKS

Status of Claims and Amendment

Claims 13 and 19 have been amended. Claims 1-12 have been canceled. Claims 13-29 are all the pending claims in the application. Claims 18 and 20-29 have been withdrawn by the Examiner as being directed to a non-elected invention. Claims 13-17 and 19 are rejected.

Claim 13 has been amended to even further clarify an isolated immunogenic, non-haemolytic *Actinobacillus pleuropneumoniae* (*App*) strain comprising at least one mutation in a transmembrane domain of the *apx*IA gene, and optionally at least one mutation in a transmembrane domain of the *apx*IIA gene, wherein only the A gene is mutated. Support for the amendment to claim 13 may be found at least at page 3, lines 23-26, sentence bridging pages 9-10, page 12, lines 26-28, and Examples of the specification.

Claim 19 has been amended to even further clarify that the porcine pleuropneumoniae is caused by *Actinobacillus pleuropneumoniae*. Support for the amendment to claim 19 may be found at least at page 1, lines 10-11 of the specification.

The specification at page 15, lines 2-14 has been amended to capitalize "Stratagene", and indicate that the vector and strains recited are trademarks, in response to an objection to the specification.

The specification at the paragraph bridging pages 5-6, the paragraph bridging pages 6-7, page 11, lines 15-20, page 15, lines 2-14, page 25, line 8, and page 26, line 27 has been amended to correct a clerical error, i.e., to italicize "*apx*."

No new matter is added.

Drawings

Applicants thank the Examiner for indicating that the drawings filed May 19, 2005, have been accepted.

Information Disclosure Statement

Applicants thank the Examiner for acknowledging the Information Disclosure Statement filed May 19, 2005, by returning a signed and initialed copy of the PTO/SB/08 form submitted therewith.

Claim of Priority

Applicants thank the Examiner for acknowledging Applicants' claim of priority to Spanish Application No. P200202663 filed November 20, 2002, as well as receipt of the certified copy of the priority document.

Brief Summary of the Invention

Actinobacillus pleuropneumoniae (App) is the causative agent of the porcine pleuropneumonia. The exotoxins of App play an important role in the infectious causes of strains of App. The main exotoxins (RTX toxins) are ApxI, ApxII, ApxIII, and ApxIV. ApxI and ApxII are haemolytic and cytolytic. (See page 1, lines 14-17 of the specification).

The genes of ApxI and ApxII are organized as operons. The operon of ApxI exotoxin contains four genes: the C, A, B, and D genes designated, respectively as, *apx*IC, *apx*IA, *apx*IB, and *apx*ID. (See page 1, lines 26-27 of specification). The operon of ApxII exotoxin contains two genes: the A and C genes designated, respectively as, *apx*IIA and *apx*IIC. (See page 2, lines 1-2 of the specification). The A gene (*apx*IA) codes for the exotoxin itself, the C gene (*apx*IC) codes for an activator protein responsible for the active conformation of the exotoxin, and the B (*apx*IB) and D (*apx*ID) genes code for membrane proteins responsible for the secretion or

transport of mature ApxI exotoxin to the external medium. (See page 1, lines 28-33 of the specification). The A and C genes of ApxII have the same roles as the A and C genes of ApxI. Id. The export of mature ApxII is due to the action of the *apx*IB and *apx*ID genes. Id.

The transmembrane domains of ApxI and ApxII exotoxins are important in the pathogenesis of the App strain because the transmembrane domains of both exotoxins form the pore in the membrane of target cells which causes osmotic imbalance leading to lysis of the target cells. (See paragraph bridging pages 3 and 4 of the specification).

The present invention relates to preparation of an immunogenic, non-haemolytic App strain in which at least one segment of the transmembrane domain of the *apx*IA and optionally of the *apx*IIA genes which codes for the haemolytic and cytolytic exotoxins are modified. There are three known transmembrane domains in ApxI and ApxII: the H1 domain, H2 domain, and H3 domain. (See page 10, lines 4-19 of the specification).

It is common knowledge that mutations or deletions in the RTX exotoxins of App lead to avirulent strains, some of which are well-characterized (Tascón et al., 1994; Jansen et al., 1995; Reimer et al., 1995)

However, strains that do not express the RTX exotoxins do not elicit a protective immune response even though they are avirulent. Accordingly, these non-immunogenic and avirulent strains cannot be used as an attenuated vaccine. The present inventors have determined that because the ApxI haemolysin is the main protective antigen of App, the corresponding gene should not be deleted to obtain a protective immune response.

The object of the present invention is to obtain immunogenic but non-haemolytic (avirulent) mutants of *Actinobacillus pleuropneumoniae*. To keep the protective immunogenicity it is necessary that the toxin (which is a porin) keep its native three dimensional

structure but lose its porin activity. In other words, the goal of the present invention is a mutant protein which is able to keep the native conformation of the immunogenic domains but which lacks its pore-forming capacity. The claimed App mutant has been obtained by the method of the present invention, which includes the steps of:

predicting the transmembrane domains of the App's RTX exotoxins (TransMem: Aloy et al., 1997; Helixmem: Eisenberg et al., 1984), and

deleting only a short stretch of amino acid residues that correspond to one of the transmembrane helices.

The present invention results in a protein that maintains its native conformation (because it is as antigenic as the wild-type) but it has lost its porin activity (conferring an avirulent phenotype to the App strains when injected in pigs). This construction has never been previously reported elsewhere, either by rational design, or by random mutagenesis.

Accordingly, the claimed mutant strain of the present invention may be used to develop a live vaccine that mimics an infection with a wild type App to induce a potent immune response but without the lesions and the signs caused by a pathogenic App strain.

The claimed mutant strain of the present invention is immunogenic and attenuated, and may thus be used as a vaccine to prevent App induced lesions and clinical signs.

Response To Elections/Restrictions

On page 2 of the Office Action, the Office Action acknowledges Applicants' election of the invention of Group I (claims 13-19) with traverse.

Specifically, the Office Action notes Applicants' argument that the invention relates to a single generic concept patentable over the prior art since the technical patentable feature is not the noted genes, but a mutation of the noted genes, especially a mutation in the transmembrane

domain of *apx*I, and optionally also, in the transmembrane domain of *apx*II, such that the strain is immunogenic and non-haemolytic.

However, the Office Action contends that this argument is not persuasive because it is the Office Action's position that Reimer et al teaches mutations in the *apx*IA and *apx*IIA genes, and the *apx*I CABD operon, and non-haemolytic strains.

Further, the Office Action states that Groups I-IV have different technical features, as they relate to different strains.

In response, Applicants respectfully request withdrawal of the Restriction Requirement because, as summarized in the Statement Substance of the Interview, filed herewith, and discussed during the Telephone Interview of May 15, 2008, the special technical feature shared by Groups I-IV is not disclosed by Reimer.

That is, Reimer does not disclose a mutant strain which comprises a mutation in at least one region of the A gene for *apx*I and optionally a mutation in at least one region of the A gene for *apx*II. Instead, Reimer discloses a wildtype strain (J45) which synthesizes and secretes exotoxins ApxI and ApxII, a mutant with the C, B, A, and D genes (*apx*ICABD operon) of ApxI completely deleted (mIT4-H), a mutant in which the deleted *apx*ICABD operon is restored (MIT4-H/pJFF800), and a mutant in which the B and D genes (*apx*IBD operon) for ApxI are restored.

Further, Applicants note that the core of the invention, i.e., an isolated immunogenic and non–haemolytic *Actinobacillus pleuropneumoniae* strain, prepared by deletion of a segment of the *apx*IA gene and optionally a segment of the *apx*IIA gene coding for a transmembrane domain of the Apx exotoxins, is novel and not disclosed by Reimer or any other, either by rational design, or by random mutagenesis.

Reimer is directed to determining the molecular level role of exotoxins ApxI and ApxII in the virulence of *Actinobacillus pleuropneumoniae* serotype 5.

Four strains of *Actinobacillus pleuropneumoniae* are disclosed at Table 1 of Reimer (see page 202), and the virulence of these strains in pigs is shown in the data at Table 3 of Reimer. Based upon Tables 1 and 3, the strains of *Actinobacillus pleuropneumoniae* described in Reimer have the following features:

(1) Strain J45 is a field isolate, which synthesizes and secretes exotoxins ApxI and ApxII, and it has strong haemolytic and cytolytic activity. It is an immunogenic strain, but virulent.

(2) Strain mIT4-H is a mutant isolated from J45 following chemical mutagenesis. Its operon *apx*ICABD is completely deleted. This *apx*ICABD operon is responsible for the synthesis, activation and secretion of exotoxin ApxI, and for the secretion of exotoxin ApxII. The mIT4-H mutant does not synthesize or export exotoxin ApxI. The mIT4-H mutant can synthesize exotoxin ApxII, but it cannot export the ApxII exotoxin. It is a non-immunogenic and avirulent strain, which is incapable of protecting pigs against subsequent challenge with the virulent parent strain J45.

(3) Strain mIT4-H/pJFF801 is a mIT4-H mutant strain that contains plasmid pJFF801. This pJFF801 plasmid restores operon *apxI*BD which is responsible for the excretion of exotoxins. This strain can synthesize and excrete exotoxin ApxII, but not exotoxin ApxI, which is mainly responsible for the haemolytic activity of *Actinobacillus pleuropneumoniae*. It is a non-immunogenic and virulent strain.

(4) Strain mIT4-H/pJFF800 is a mIT4-H mutant strain that contains plasmid pJFF800. This pJFF800 plasmid restores operon *apx*ICABD, and the strain can synthesize and secrete

exotoxins ApxI and ApxII. The extracelular haemolytic activity is equal or higher than the virulent parent strain J45. It is an immunogenic and virulent strain.

Thus, the claimed isolated immunogenic, non-haemolytic (avirulent) App strain of the present invention is not disclosed or suggested in Reimer because:

(a) strains J45 and miT4-H/pjFF800 have the whole genetic information and they are virulent strains,

(b) strain mIT4-H is a non-immunogenic and avirulent chemical mutant, and

(c) strain mIT4-H/pJFF801 has genetic modifications and it is non-immunogenic and virulent.

In addition, and as discussed during the Telephone Interview of May 15, 2008, Applicants have amended claim 13 to recite that only the A gene is mutated in order to advance withdrawal of the Restriction Requirement, which is respectfully requested.

Response To Objections To The Specification

On page 3 of the Office Action, the Office Action objects to the disclosure because "Strategene" is not capitalized, and indicated as a trademark.

In response, Applicants have amended the specification at page 15 to capitalize the term "Stratagene", and indicate that the vector and strains recited at page 15 are trademarks.

Withdrawal of the grounds of objection is respectfully requested.

Response To Claim Rejections Under 35 U.S.C. § 101

Claims 13-17 are rejected under 35 U.S.C. § 101, as being allegedly directed to non-statutory subject matter. Specifically, the Office Action contends that the mutations in the recited genes can be found in nature. The Office Action suggests amending claim 13 to refer to

an "isolated" immunogenic, non haemolytic Actinobacillus pleuropneumoniae (APP) strain in order to overcome the rejection .

In response, Applicants have amended claim 13 to recite "isolated" as suggested by the Office Action.

Withdrawal of the rejection under § 101 is respectfully requested.

Response To Claim Rejections Under 35 U.S.C. § 112

Claim 19 is rejected under §112, first paragraph because while the specification is asserted by the Office Action to be enabling for an immunogenic, non-haemolytic Actinobacillus pleuropneumoniae strain comprising a mutation in at least one region of the *apx*IA, and optionally a mutation in at least one rejection of the *apx*IIA gene, such does not provide enablement for a vaccine, as claimed.

The Office Action appears to assert that Applicants' specification is not enabled for the prevention, amelioration, or treatment of all infectious diseases, and one of ordinary skill in the art would not be enabled to make and/or use the claimed invention without undue experimentation. In this regard, the Office Action appears to assert that the specification does not provide sufficient evidence that the claimed vaccines are capable of inducing protective immunity. Also, the Office Action appears to assert that the specification provides insufficient guidance to one of ordinary skill in the art on how to obtain the claimed vaccine because the purification steps to obtain the claimed vaccines is not evident.

Thus, because allegedly the amount of direction of guidance presented in the specification is limited only to production of *apx*IA and *apx*IIA, there is insufficient guidance, e.g., working examples, as to how to the vaccine composition can prevent and treat a disease, and the skill required of one of ordinary skill in the art is high, i.e., post-doctoral level, the Office

Action concludes that the specification does not enable one of ordinary skill in the art to make or use the claimed invention.

Initially, Applicants note that the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. M.P.E.P. § 2164.01. Also, compliance with the enablement requirement does not turn on whether an example is disclosed, and the specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it. M.P.E.P. § 2164.02.

Applicants note that the specification is enabling for making the claimed vaccine composition of the present invention because as discussed in WO97/16532A1 and EP810283A2 (see Background section of the specification at page 2, lines 10-19), the methods for making and using a vaccine strain are commonly known and used in the art. Also, as discussed in the Ellis, R.W. (Vaccines, Chapter 29, Poltkin et al. (Eds.) (W.B. Saunders, Philadelphia, 1988) reference cited at page 6 of the Office Action, the technology for making live, attenuated vaccines use "routine techniques in cell culture" and is "enabling [for] scientists to design attenuated vaccines" (see page 568, 2nd column of Ellis).

Further, Applicants note that it is commonly known in the art that the preparation of a vaccine comprising a live, attenuated strain does not involve any protein purification process. Because the claimed vaccine is prepared from live, attenuated App strains, the vaccine may be prepared directly from the live App attenuated strains by suspending the live, attenuated App strains in a pharmaceutically acceptable vehicle, e.g., aqueous-type fluid.

In addition, Applicants submit herewith a Rule 132 Declaration demonstrating that the claimed vaccine composition comprising the mutant App strain of the present invention is useful as a vaccine against *Actinobacillus pleuropneumoniae* that causes porcine pleuropneumoniae.

As shown in the Rule 132 Declaration, the live, attenuated vaccine produced from the claimed immunogenic, non-haemolytic *Actinobacillus pleuropneumoniae* strain of the present invention is useful and effective as a vaccine against *Actinobacillus pleuropneumoniae* that causes porcine pleuropneumoniae. Specifically, the experimental data demonstrates the efficacy of the vaccine strain CECT 5994 (see page 4, lines 15-17, and page 13, lines 22-28 of the specification), obtained from the mutation (deletion) of a segment of the *apx*IA gene and additionally a segment of the *apx*IIA gene that codes for the second transmembrane domain of exotoxins, ApxI and ApxII, respectively. CECT 5994 provides an immunogenic and non-haemolytic effect to provide protection of swine against the strain of *Actinobacillus pleuropneumoniae* that causes porcine pleuropneumoniae.

Applicants also submit herewith a biological receipt of deposit for CECT 5985 (claim 20) and a biological receipt of deposit for CECT 5994 (claim 22)[1], both made under the Budapest Treaty, to further demonstrate that the specification enables one of ordinary skill in the art to make and use Applicants' claimed invention.

Reconsideration and withdrawal of the rejection under §112, first paragraph, is respectfully requested.

[1] Copies of the original biological receipt of deposits for CECT 5985 and CECT5994, and the English translation of these original biological receipt of deposits are submitted herewith.

Response To Claim Rejections Under 35 U.S.C. § 102(b)

1. Claims 13-17 and 19 are rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 6,019,984 to MacInnes et al. ("MacInnes").

Specifically, the Office Action asserts that MacInnes teaches deletion mutations, *apx*IA and *apx*IIA (claims 6-12 and columns 3 and 4 and Figures of MacInnes).

The Office Action acknowledges that MacInnes does not explicitly teach nucleotides 886 to 945 of *apx*IA gene.

In response, Applicants note that MacInnes does not explicitly or inherently disclose the presently claimed invention.

MacInnes is directed to a method of preparing a vaccine in which the microorganism has at least one RTX toxin which is substantially cell-associated. However, "substantially cell-associated" as defined at column 8 in MacInnes is not a mutation. Also, even though MacInnes discloses a modified App strain, only the B and D genes is modified (column 30 and claims 6-12 of MacInnes). MacInnes does not disclose at least one mutation in the transmembrane domain of the A gene of *apx*I or optionally *apx*II.

In contrast, the presently isolated immunogenic, non-haemolytic *Actinobacillus pleuropneumon*iae (*App*) strain comprises at least one mutation in a transmembrane domain of the *apx*IA gene, and optionally at least one mutation in a transmembrane domain of the *apx*IIA gene, wherein only the A gene is mutated.

Reconsideration and withdrawal of the rejection under § 102(b) is respectfully requested.

2. Claims 13 15, 17 and 19 are rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 6,472,183 to Prideaux et al. ("Prideaux").

Specifically, the Office Action asserts that Prideaux teaches deletion mutations, *apx*IA and *apx*IIA (see claims 1-4 and columns 3 and 4 of Prideaux).

In response, Applicants note that Prideaux does not explicitly or inherently disclose the presently claimed invention.

Prideaux is directed to a modified APP strain comprising an RTX A gene and an inactivated RTX C gene, so that the C gene is mutated. Prideaux does not disclose at least one mutation in the transmembrane domain of the A gene of *apx*I or optionally *apx*II.

In contrast, the presently isolated immunogenic, non-haemolytic *Actinobacillus pleuropneumon*iae (*App*) strain comprises at least one mutation in a transmembrane domain of the *apx*IA gene, and optionally at least one mutation in a transmembrane domain of the *apx*IIA gene, wherein only the A gene is mutated.

Reconsideration and withdrawal of the rejection under § 102(b) is respectfully requested.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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